Effects of vaccination with F-strain *Mycoplasma gallisepticum* on egg production and quality parameters of commercial layer hens previously vaccinated with 6/85-strain *Mycoplasma gallisepticum*[1]

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ABSTRACT This study was conducted to determine the effect of overlaying (revaccinating) F-strain *Mycoplasma gallisepticum* at 22 or 45 wk of age on commercial leghorn hens previously vaccinated with 6/85-strain *M. gallisepticum* at 10 wk of age. The treatment groups included unvaccinated hens (group 1), hens receiving 6/85-strain *M. gallisepticum* only (group 2), and hens receiving 6/85-strain *M. gallisepticum* followed by F-strain *M. gallisepticum* at either 22 (group 3) or 45 (group 4) wk of age. There was no significant effect on egg production or egg size distribution between any of the treatment groups, unlike previous studies looking at F-strain vaccination only. Egg quality parameters, including eggshell strength, Haugh unit score, and blood-meat spot were similar between the different treatment groups. There was a difference in the rate of pimpling at postpeak production for the treatment group receiving F-strain *M. gallisepticum* at 22 wk of age, consistent with previously published results. This work suggests that hens previously vaccinated with 6/85-strain *M. gallisepticum* can be safely revaccinated with F-strain *M. gallisepticum* to increase protection from field strains while ameliorating the adverse effects associated with F-strain *M. gallisepticum* vaccination in layers post onset of lay.

Key words: *Mycoplasma gallisepticum*, vaccination, layer, egg production

2010 Poultry Science 89:501–504
doi:10.3382/ps.2009-00519

INTRODUCTION

Mycoplasma gallisepticum is an infectious agent of poultry, including both layer and broiler chickens as well as turkeys and other avian species (Evans et al., 2005). Although increased flock mortality is one outcome of *M. gallisepticum* infection, most often, the result is decreased egg production, decreased carcass weight, and increased carcass condemnation (Ley, 2003). Control of *M. gallisepticum* infection for meat-type flocks is carried out through biosecurity and the all-in-all-out management of the rearing facility. Multiage complexes, including layer complexes and breeding facilities, cannot depend on the all-in-all-out control method and must depend on biosecurity and other methods for *M. gallisepticum* control including vaccination.

Three live *M. gallisepticum* vaccines are currently available for layer chickens. The vaccines are based on 3 different *M. gallisepticum* strains: F strain (F VAX-MG, Intervet Schering Plough Animal Health, Boxmeer, the Netherlands; Poulvac Myco F, Fort Dodge Animal Health, Fort Dodge, IA), 6/85 strain (Mycovac-L, Intervet Schering Plough Animal Health, Millsboro, DE), and ts-11 strain (*M. gallisepticum* vaccine, Merial Select, Duluth, GA) (Evans et al., 2005; Kleven, 2008). Initial studies showed that vaccination with F strain may decrease egg production compared with unvaccinated controls, but the decrease was less than if the chickens were infected with field *M. gallisepticum* strains (Carpenter et al., 1981). However, it has also been reported that egg losses might not be apparent under commercial conditions (Evans et al., 2005). In addition, F-strain vaccination may also lead to shifting in egg sizes produced from larger to smaller egg sizes, most notably from large to medium egg size (Branton et al., 1999). Vaccination with either 6/85 or ts-11 strains does not result in the decreased egg production or shifts in egg size that may be seen with F-strain *M. gallisepticum*; however, they also do not provide the same level of protection against challenges with field strains (Abd-el-Motelib and Kleven, 1993; Evans et al., 2007; Kleven, 2008). The lower level of protection afforded by these vaccines as compared with the F strain

Received October 21, 2009.
Accepted November 23, 2009.

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

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suggests the possibility of needing to revaccinate with F strain if either 6/85 or ts-11 strains are insufficient to control *M. gallisepticum* in an infected flock. This has led to the question of what effects may be seen in a flock previously vaccinated with one of these milder *M. gallisepticum* vaccine strains if they are subsequently vaccinated with the F-strain *M. gallisepticum.* The present study was designed to address this question by looking at the effect on egg production and egg quality parameters from commercial layer hens that were either unvaccinated, vaccinated with the 6/85 strain at 10 wk of age, or vaccinated with the 6/85 strain at 10 wk of age followed by F-strain *M. gallisepticum* at either 22 or 45 wk of age.

MATERIALS AND METHODS

Housing and Management

One-day-old Hy-Line W-36 Leghorn pullets obtained from a commercial source were used in each of 2 trials. Pullets were housed on dry pine shavings before the experiment began, as described previously (Burnham et al., 2002). At 7 wk of age, pullets were screened by serum plate agglutination (**SPA**) test and were shown to be free of *M. gallisepticum.* The choanal clefts of 20 pullets were also swabbed and cultured for *Mycoplasma* growth (Branton et al., 1984) and were shown to be negative. At 10 wk of age, pullets were randomly assigned to treatment groups and placed in isolation units with 11 pullets per isolation unit (Branton and Simmons., 1992). At onset of lay (approximately 20 wk of age), the number of pullets per box was reduced from 11 to 10.

Pullets were randomly divided into 4 treatment groups with individual isolation units serving as the treatment group. Designation of isolation units to treatment groups was made to prevent cross-contamination between the treatment groups. For each trial, chickens were provided ad libitum access to both feed and water as described previously (Branton et al., 2002). Five diets were provided over the course of each trial as follows: 0 to 6 wk, starter; 6 to 12 wk, grower; 12 to 18 wk, developer; 18 wk to onset of lay, prelay; and onset of lay to conclusion of experiment, layer. These diets were formulated to meet NRC recommendations and have been described previously by Burnham et al. (2002). Temperature was maintained at 23°C for the duration of the experiment. Through 18 wk of age, lighting was maintained at 10 h per day. At 18 wk of age, the lighting duration was increased 15 min per week until a duration of 16 h and 15 min was achieved as described previously (Branton et al., 2002).

Mycoplasma Vaccination

Treatment groups used were as follows: control (no *M. gallisepticum* exposure); 6/85-strain *M. gallisepticum* (Mycovac-L, Intervet Schering-Plough Animal Health at 10 wk; 6/85-strain *M. gallisepticum* (Mycovac-L) at 10 wk, F-strain *M. gallisepticum* at 22 wk; and 6/85-strain *M. gallisepticum* (Mycovac-L) at 10 wk, F-strain *M. gallisepticum* at 45 wk. Vaccine was administered by fine spray to groups of 11 pullets confined in small coops. The rehydrated 6/85-strain *M. gallisepticum* vaccine was also cultured to ensure viability and for each trial was found to have a titer of approximately 1×10^9 cfu/mL. At 22 and 45 wk, layers from either the 22-wk treatment group or the 45-wk treatment group were removed from their respective isolation units and vaccinated with 0.04 mL of overnight culture of high-passage (99th passage above the unknown passage level) F-strain *M. gallisepticum* in Frey's medium in the left eye, as described previously (Branton et al., 1997). Titers were determined to be 5.2 and 4.0×10^8 cfu/mL for the 22-wk vaccination and 3.0 and 2.8×10^9 cfu/mL for the 45-wk vaccination per 40-µL dose for trials 1 and 2, respectively.

Data Collection

Eggs were collected and production data were recorded daily for each isolation unit for the duration of the experiment. Eggs collected on Tuesdays and Wednesdays were tested for eggshell strength, scored for Haugh units, and scored for the incidence of pimpling, and blood-meat (**B-M**) spots. Eggshell strength was measured using a stress-strain measuring instrument as described previously (Reece and Lott, 1976). Haugh units were scored using a Model EQM egg quality management system (Technical Services and Supplies Limited, York, UK). Pimpling and B-M spots were scored as either yes (1) or no (0) for each as described previously (Branton et al., 1988). Egg size classes were determined by egg weights as described previously (Branton et al., 2002).

Statistical Analysis

The study was conducted using a randomized design with trial as block. The data were analyzed in accordance with the time of F-strain *M. gallisepticum* vaccination. Weeks 23 through 44 (time 1) and wk 45 through 52 (time 2) were analyzed separately. Two trials were performed and the data from both trials were combined for analysis and reporting. Time 1 consisted of 3 treatments: control, 6/85 only, and 6/85 with F-strain *M. gallisepticum* at 22 wk with 4 replicates of each per trial. Time 2 consisted of the 3 treatments from time 1, plus a fourth treatment, 6/85 with F-strain *M. gallisepticum* at 45 wk. Isolation units were considered the experimental treatment group, and all results from within individual isolation units were averaged on a weekly basis before analysis. Blood and meat spot data were added together, and the weekly data for pimpling and B-M spot were averaged across the 2 time intervals for each experimental unit before analysis. All data were $\log_{10}$-transformed and analyzed using the

Table 1. The effect of 6/85-strain vaccination at 10 wk of age and overlay with F strain at 22 and 45 wk of age on hen-day egg production, eggshell strength, Haugh units, pimpling, and blood-meat (B-M) spot incidence

	Hen-day egg production (%)		Eggshell strength (kg)		Haugh unit		Pimpling[1]		B-M spot incidence[1]	
Item	23 to 44 wk	45 to 52 wk	23 to 44 wk	45 to 52 wk	23 to 44 wk	45 to 52 wk	23 to 44 wk	45 to 52 wk	23 to 44 wk	45 to 52 wk
Control	83.17	78.21	3.3	3.1	95.87	91.44	0.028	0.076[b]	0.0059	0.0083
6/85	79.2	75.25	3.12	3.14	96.65	92.58	0.026	0.065[b]	0.0049	0.0037
6/85, F strain at 22 wk	81.62	79.06	3.3	3.05	96.43	92.66	0.04	0.12[a]	0.0043	0.0056
6/85, F strain at 45 wk		77.25		3.08		92.77		0.072[b]		0.0048
SEM[2]	0.52	0.68	0.0096	0.014	0.16	0.17	0.0048	0.0095	0.0014	0.00086

[a,b]Values within a column without common superscripts are significantly different ($P \leq 0.05$).

[1]Incidence per egg.

[2]Standard error of the mean based on pooled estimate of variation.

repeated measures procedure of SAS Analyst except pimpling and B-M spot, which were analyzed using the mixed procedure of SAS Analyst (SAS Institute, 2003). Where applicable, differences in results were compared using least squares means (Tukey) with $P \leq 0.05$ considered significant.

RESULTS

All pullets were shown to be SPA-negative for *M. gallisepticum* infection at 7 wk, before the start of the experiment. Choanal cleft swabs taken from twenty 7-wk-old pullets were cultured for 30 d, and no *Mycoplasma* growth was observed. Serum samples obtained at the end of the experiment showed a strong positive response to F-strain *M. gallisepticum* vaccination (3+ SPA score). Chickens receiving the 6/85 strain only had a much lower negative serum response (SPA score of 0 to 1). Control birds had no detectable serum response (SPA score of 0).

No significant differences were observed among the treatment groups for mean hen-day egg production, eggshell strength, or Haugh units for wk 23 to 44 or wk 45 to 52 (Table 1). Analysis of eggs for pimpling incidence did show a significant difference among the treatment groups. Eggs from hens receiving F-strain *M. gallisepticum* at 22 wk had a significantly higher incidence of pimpling compared with the other treatment groups during the later stages of the laying cycle, wk 45 to 52 (Table 1). There were no significant differences among the different treatment groups for B-M spots.

The egg size results for the different treatments did not show any statistical difference when compared among treatment groups or within treatment groups compared with age for either wk 23 to 44 or wk 45 to 52 (Table 2). There were notable differences in the numbers of eggs produced for certain sizes in each of the 2 time categories, including differences in the number of jumbo eggs as well as the number of small eggs from wk 45 to 52, suggesting differences may actually occur between those groups. However, the small number of eggs for those size groups limits the ability to analyze data from those groups.

DISCUSSION

Results from this work are consistent with results previously reported for chickens receiving 6/85- or F-

Table 2. Egg size distribution of eggs collected 2 d each week for wk 23 to 44 and 45 to 52 for control hens or hens vaccinated with 6/85 at 10 wk or vaccinated with 6/85 at 10 wk of age and overlaid with F strain at 22 or 45 wk of age

	Jumbo		Extra large		Large		Medium		Small		Peewee		Undergrades	
Item	No.[1]	%[2]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Weeks 23 to 44														
Control	11	0.39	70	2.48	810	28.67	1,464	51.82	452	16	17	0.6	1	0.04
6/85	12	0.44	83	3.06	796	29.38	1,403	51.79	402	14.84	11	0.41	2	0.07
6/85, F strain at 22 wk	4	0.14	77	2.75	766	27.31	1,480	52.76	456	16.26	22	0.78	0	0
SEM[3]		0.025		0.0054		0.0092		0.0085		0.013		0.014		0.0081
Weeks 45 to 52														
Control	4	0.42	121	12.72	577	60.67	237	24.92	12	1.26	0	0	0	0
6/85	7	0.76	119	12.96	576	62.75	214	23.31	2	0.22	0	0	0	0
6/85, F strain at 22 wk	2	0.21	118	12.28	540	56.19	290	30.18	10	1.04	1	0.1	0	0
6/85, F strain at 45 wk	3	0.32	136	14.42	545	57.79	257	27.25	2	0.21	0	0	0	0
SEM[3]		0.0032		0.0077		0.0097		0.0097		0.014		NA[4]		NA

[1]Number of eggs laid.

[2]Percentage of total eggs laid.

[3]Standard error of the mean based on pooled estimate of variation.

[4]NA = not applicable.

strain *M. gallisepticum*. In those previous reports, no significant difference was seen in hen-day egg production, eggshell strength, or Haugh units (Branton et al., 1997, 2002). The overlay (revaccination) of one vaccine strain onto hens already vaccinated with a different *M. gallisepticum* strain did not change those results. One study showed that vaccination of *M. gallisepticum*-clean hens with F strain at 45 wk of age led to decreases in egg production and increases in Haugh unit score (Branton et al., 1988). The present work suggests that prior vaccination with 6/85 strain ameliorates these effects.

The increase in pimpling incidence for hens vaccinated with F strain at early stage egg production (22 wk) versus postpeak production (45 wk) is consistent with earlier results. Previous work has shown that vaccination with F strain at 45 wk did not lead to an increase in pimpling incidence, whereas vaccination with F strain at 10 wk of age did lead to an increase in pimpling incidence (Branton et al., 1988, 1997). Both pimpling incidence and eggshell strength are regarded as measures of uterine function. Neither 6/85-strain nor F-strain *M. gallisepticum* has been shown to influence eggshell strength (Branton et al., 1988, 1997, 2002). Because vaccination with the 6/85 strain before F-strain vaccination does not protect from increased pimpling incidence, this suggests that either F strain affects uterine function using a mechanism not present in the 6/85 strain or that the immune response to the 6/85 strain does not provide sufficient protection for the uterus. These results indicate that revaccination with F-strain *M. gallisepticum* for hens previously vaccinated with 6/85-strain *M. gallisepticum* provides a viable method for increasing resistance to *M. gallisepticum* infection while ameliorating the decrease in egg production associated with F-strain *M. gallisepticum* vaccination during the laying cycle.

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